5/25/2001, A.H.

STREAKING OUT OF CLONES AND INOCULATING FOR MINIPREPS

See Astrid Haugen for Plate/Row/Column locations of clones used in Microarray center.

1) Take out appropriate 96-well bacterial culture plate.
2) Put on dry ice. Do not thaw.
3) Use a sterile pipette tip or toothpick to touch appropriate clone.
4) Streak out on an agar plate made with LB broth and 50 µg/ml Ampicillin. Drop the tip or toothpick immediately into 5 ml of the same liquid broth in a 50 ml conical tube.
5) Set the streaked out agar plates overnight in an incubator at 37°C and the liquid cultures in a shaking incubator at 37°C/225 RPM.
6) Take out plates the next morning and put parafilm around them and store in the refrigerator at 4°C.
7) Remove miniprep cultures from the shaking incubator as early in the day as possible.
8) Do minipreps. Once DNA isolation proves positive, distribute agar plate streaks to the appropriate investigator.

MINIPREPS

This protocol uses the Qiagen Miniprep Plasmid Purification Kit with some modification.

1) Pipette 1.6 ml resuspended culture into an eppendorf tube. Spin down at high speed in a microcentrifuge.
2) Decant the supernatant. There should be about 50 µl residual broth in the tube along with the pelleted cells. Vortex intensely to resuspend the pellet in the left over broth.
3) Add 250 µl Buffer P1 (make sure the kit's RNAse A has been added to the buffer).
4) Add 250 µl of Buffer P2 and gently invert the tube 4-6 times. Do not vortex or shearing may occur. Do not allow lysis to proceed more than 5 minutes.

5) Add 350 µl Buffer N3 and invert the tube immediately but gently, 4-6 times. The solution should become cloudy.
6) Centrifuge for 10 minutes. A compact white pellet will form.
7) During centrifugation, place a QIAprep spin column in a 2-ml collection tube.
8) Apply the supernatants from step 6 to the QIAprep column.
9) Centrifuge 30-60 seconds. Discard the flow-through.
10) Wash the QIAprep spin column by adding 0.5 ml of Buffer PB and centrifuging 30-60 seconds. Discard the flow-through.
11) Wash the QIAprep spin column by adding 0.75 ml of Buffer PE and centrifuging 30-60 seconds.
12) Discard the flow-through, and centrifuge for an additional 1 minute to remove the residual wash buffer. This is important to remove as much of the residual ethanol as possible.
13) Place QIAprep column in a clean 1.5 ml eppendorf tube. To elute the DNA, add 50 µl of Buffer EB or water to the center of each QIAprep column, let stand for 1 minute, and centrifuge for 1 minute.
14) Prepare a 1X – 1.0% TBE agarose gel by melting 1 gram Agarose in 100 ml of 1X TBE. Melt in a microwave, shaking it every once in a while. Be sure it all melts in solution. Beware of over-boiling.
15) Add 1 µg/µl Ethidium Bromide (2.5 µl 10mg/ml Ethidium Bromide in 25 ml of melted agarose – MUTAGEN, USE GLOVES).
16) Pour melted agarose into appropriate mini-gel former and allow the gel to solidify. Make sure the appropriate sized comb is inserted in the gel.
17) When the gel is hard, remove all gel formers. Add 1X TBE buffer so that the gel is covered. Remove comb carefully.
18) Take 3-5 µl uncut DNA and add 2 µl loading dye. Mix in tube and spin.
19) Load 6 µl in small wells. Don’t forget to run a 1kb marker next to the samples. DNA will migrate toward the positive charge, so make sure the electrodes are positioned appropriately. Run at 100 volts, until the bromophenol blue dye starts getting to the end.
20) Take a picture of the gel as described in appropriate SOP.

21) If samples look good (you have DNA), then prepare digests. Cut with at least EcoR1 to make sure the DNA cuts and is free of contamination.
22) Take 5 µl of uncut DNA, unless the uncut looks weaker (then cut more). Add appropriate enzyme buffer so that it is 1X. A total volume of 10 µl – 20 µl is good. Add 1 µl of 10 U/µl EcoR1 enzyme. Mix, spin down, and place in a 37oC incubator or waterbath overnight.
23) Run on a gel the next day, the same way as uncuts were run.
24) Digests may also be done in two hours as well, if there is time.